Epidermal DNA Repair Under Repeated Exposure Conditions is Complex

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We thank Hemminki and Snellman (2002) for their interest in our studies and we are pleased to be able to clarify the issues they raise. The main purpose of our study (Sheehan *et al* 2002), as indicated by the title of the paper, was to determine the photoprotective properties of induced pigmentation by solar simulated radiation (SSR) in skin types II and IV. We used individual biological [just perceptible minimal erythemal doses (MED)] dose increments rather than fixed physical (J/cm2) doses because individual exposure in real-life is determined by sun-sensitivity. This is supported by recent studies, in which personal exposure has been electronically monitored over 114 days, that show that skin types IV have about 40% more solar UVR-exposure than skin types II (Thieden E. & Wulf H.C., personal communication).

Photoprotection against DNA photodamage from 2 MED challenge dose of SSR was assessed one week after the last tanning treatment because we expected, based on work in our laboratory (Young *et al*, 1996) and that of Hemminki (Bykov *et al*, 1999), to find that the majority of the thymine dimers accrued during the tanning protocol would have been removed by that time.

The essential point of Fig 3 (Sheehan *et al* 2002) is that there was a highly significant reduction of thymine dimers in skin type IV one week after the last treatment, but a trivial nonsignificant reduction in skin type II. In support of our observations, we would like to have been able to cite Hemminki *et al* (2002), who show the persistence of 2.4% and 8.9% of thymine dimers in skin types I and IV, respectively, for up to three weeks after a single SSR exposure, but unfortunately this paper was published in April 2002, one month before the publication of our paper that was accepted for publication in October 2001. Our study shows the persistence of dimers in skin type II for one week after two weeks daily (Mon-Fri) SSR exposure and, to a much lesser extent, the persistence of dimers in skin type IV. Thus, both groups, using different techniques and irradiation protocols, show that epidermal thymine dimers persist.

Hemminki and Snellman (2002) comment on the lack of a dose–response effect in skin type IV in Fig 2(a) (Sheehan *et al* 2002). An analysis of the individual data shows that all skin type II volunteers show a dose–response, whereas this is only seen in 4/6 of skin type IV volunteers, which reduces the mean. Nonetheless, we have observed this plateau effect with SSR before (Young *et al*, 1996) and fully recognize that immunohistochemical techniques are semiquantitative. There are two dose–response curves for thymine dimers in publications from Hemminki's laboratory. One, *in vivo* with SSR (Bykov *et al*, 1998a), does indeed show a very linear dose–response but the other, in skin explants with maximum UVC dose of about 2–3 MED (Bykov and Hemminki, 1995), shows an aysymptotic response similar to ours, even though a straight line has been fitted through the data.

Hemminki and Snellman (2002) contend that antibody techniques are invalid and suggest that our results may be due to nonspecific background staining. All sections were stained as one batch because we are aware that different staining runs may give different results. We have negative controls for no SSR exposure in all cases, and we find that the antibody we used shows specific and discrete nuclear staining with virtually no background staining at all. For example, see Fig 4(a) in Young *et al* (1998a) that shows dimer positive nuclei in the supra-basal nuclei of the epidermis after 290 nm but no staining at all in the basal layer of the same section. In contrast Fig 4(b) and 4(c) show staining throughout the epidermis and dermis after irradiation with 300 nm.

This differential staining on the same skin section shows the great advantage of localizing DNA photodamage within the epidermis, dermis, and even within specific cell types such as melanocytes (Young *et al*, 1998b). The techniques described by Hemminki and Snellman (2002) require the disruption of the epidermis and are totally unable to provide any information whatsoever on localization or cell type specificity. For example, persistent dimers in the suprabasal epidermis are unlikely to have any biological consequence. It is our view that the understanding of the biological significance of DNA repair in human skin is best served by the use of a variety of different techniques. Hemminki and Snellman recognize this because as coauthors of Xu *et al* (2000a), they state "Because melanocytes only make up 5%-10% of the cell population only immunohiostochemical techniques would allow a direct assessment of DNA damage and repair in melanocytes *in situ*" (Young *et al*, 1996, 1998a).

Hemminki and Snellman's (2002) argument that our data are invalid also assumes that the DNA repair response after repeated exposure is the same as the response after a single exposure, which does not allow for the possibility of modification of the repair response by repeated exposure. In fact, various factors are likely to be important and the level of DNA photodamage at a given time is

Manuscript received June 3, 2002; accepted for publication July 11, 2002

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likely to be a complex balance of individual repair at 24 h, apoptosis, necrosis, the addition of damage from a subsequent exposure 24 h later, epidermal proliferation, the loss of DNA damage through desquamation that is likely to be accelerated by repeated SSR exposure, as evidenced by stratum corneum thickening, and induced photoprotection. The dynamics of these complex interactions are very likely to be time-dependent, and it is possible that a steady homeostatic state may be reached.

We note that Bataille *et al* (2000) (of which Hemminki is the senior author) published a study in which epidermal DNA photodamage was assessed in the noninvolved skin of psoriatics undergoing UVB phototherapy. The abstract of this paper states "Overall, the levels of TT = T and TT = C reached a plateau at three exposures and were found to decrease with subsequent exposures despite increasing UVB doses" (there were no data from a single exposure) and "No association was found between challenge dose of UVB and photoproduct yield in this study." The arguments Bataille *et al* (2000) used to explain their results are largely similar to those that we give in the paragraph above.

In support of our observations and conclusions we refer to a recently completed similar study in skin type II with a different thymine dimer antibody (Kamiya Biomedical Company, Seattle, WA), and in which we counted dimer positive cells in different epidermal layers as opposed to mean optical density per nucleus. This study has given virtually identical results to those of Sheehan *et al* (2002). However, it also included a biopsy taken at the end of week 1 (5 × 0.65MED) which showed two-fold increase of basal cell dimer positive nuclei when compared with a single 0.65MED exposure, but at the end of week 2 (10 × 065MED) the number of dimer positive cells was similar to that of a single 0.65MED dose. Interestingly, this reflects the skin type II erythema time-course of **Fig 1*a*** in Sheehan *et al* (2002) which shows an increase over the first week with a reduction in a plateau in week two. In this context it is worth noting that xeroderma pigmentation (XP) patients, known to have poor DNA repair, show persistent erythema (Berg *et al*, 1998).

We have a significant amount of as-yet unpublished data which shows that acute SSR responses may not predict the cellular, apoptotic, and immunological responses from repeated SSR exposure, and that an acute response may be different on untreated skin compared with repeatedly irradiated skin, and that this difference varies considerably with endpoint. We believe that skin has varying adaptive responses other than optical protection by tanning.

One of the reasons for our joint study with Hemminki (Bykov *et al*, 1999) was to verify our conclusions on repair of cyclobutane pyrimidine dimers and 6–4 photoproducts in an earlier study (Young *et al*, 1996) using antibody techniques. Both studies, using 2 MED of the same source of SSR, showed rapid repair of 6–4 photoproducts and much slower repair of CPD. Young *et al* (1996) estimated that the mean half-life of thymine dimers in skin type I + II was 33.3 h. Bykov *et al* (1998a) estimated that 50% of thymine dimers were removed after 32 h after a single exposure to SSR (skin type not specified). More recently, Bykov *et al* (1999) showed a 50% reduction at 17.2 h in skin type II & Xu *et al* (2000b) show data (Fig 3) that suggest a 50% reduction of thymine dimers at about 42 h that is skin type independent. The main conclusion that can be reached on thymine dimer repair is that it is relatively slow and shows very large interperson variation. We also observe large interperson variation in thymine dimer repair capacity. Thus, different results are likely with different small groups of human volunteers.

We fully endorse the development of reliable quantification of DNA photodamage *in situ* but are puzzled by the unaccountably large interpersonal variation within a given skin type grouping when a fixed UVR dose is given. Such large differences (ranging from 5 to 30 fold in skin types II/III and I/II, respectively) (Bykov *et al*, 2000, 1998b, respectively) on the lower back cannot possibly be due to variation in constitutive pigmentation related photoprotection, as suggested by Bykov *et al* (2000), because Xu *et al* (2000b) have shown that the difference in photoproduct levels from a fixed SSR dose on previously unexposed buttock skin in skin types I/II and III/IV is not significant for 3 types of DNA photolesion (including thymine dimers) and only 1.4 fold greater in skin types I/II for thymine-cytosine CPD. Hemminki *et al* (2002) show that thymine dimers are induced at a significantly higher level (~ × 2) on unexposed buttock skin in one study group of skin type I compared with type IV after a fixed dose of SSR, yet in a larger but older study group of skin type I there was no difference between skin types I and IV. Does this mean that older unexposed buttock skin becomes less sensitive to DNA photodamage? It seems improbable that large interperson variation could be due to any facultative pigmentation on the lower back, especially > 30-fold variation in skin types I/II (Bykov *et al*, 1998b). Indeed, Hemminki *et al* (1999) have shown that the DNA photodamage protection factor of UVA-induced pigmentation in fair skinned people is 1.2 but this complete lack of protection is not surprising given the well-known differences between UVA and UVB induced pigmentation. A review of pigmentation-related protection factors against DNA photodamage (Young and Sheehan 2001) shows that they are probably in the region of 2–3, which cannot explain large interperson variation of DNA damage induction within a given skin type. We also note that there is a ~ 30% – ~56% variation in repeated analysis of the same samples (Bykov *et al*, 1998b; Hemminki *et al*, 1999) and cannot help but wonder if this contributes to the somewhat contradictory results given above.

In conclusion, we endorse quantitative techniques but feel that immunostaining has an important role in the assessment of DNA photodamage and its repair in human skin, and we totally disagree with Hemminki and Snellman (2002) that quantitative and qualitative data obtained with such techniques are unreliable, all the more so when many of the biological conclusions that we have reached with our techniques are in broad agreement with those from Hemminki's laboratory.

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